REMARKS

The Examiner is thanked for the due consideration given the application. An appendix containing specification sheets is attached to this paper.

Claims 19-25, 27-34 and 36-42 are pending in the application. Claims 27 and 36 have been amended to not depend on a canceled claim. Claim 33 has been amended to clarify the language in a non-narrowing fashion that raises no new issues. The independent claims have also been amended to set forth that the at least one nucleotide in the range of 1-50 mole-% is present to avoid quenching and intra-molecular thiol group formation, which finds support in the specification at, e.g., page 15, lines 14-17.

No new matter is believed to be added to the application by this amendment.

Entry of this amendment under 37 CFR §1.116 is respectfully requested because is complies with a matter of form set forth in the Office Action and addresses issues that will place the application in condition for allowance or, alternately, place the application in better form for appeal.

Claim Objections

Claims 27 and 36 have been objected to as being dependent upon canceled claims. Claims 27 and 36 have been amended to not depend upon canceled claims.

The Specification/Rejection Under 35 USC §112, First Paragraph

The specification has been objected to as containing new matter. In a linked issue, claims 32 and 41 have been rejected under 35 USC §112, first paragraph as failing to comply with the written description requirement. This rejection is respectfully traversed.

In the previous response, the specification and claims were amended to set forth generic terminology for such materials as NP-40, Tween 20 and Triton X-100.

The applicant believes that these materials are notoriously well known, and the introduction of generic terminology does not equate to new matter.

As evidence thereof, attached to this paper are typical specification sheets for these materials, which clearly set forth the chemical constituents of NP-40, Tween 20 and Triton X-100.

Therefore, there has been no new matter introduced to the application.

Withdrawal of the objection to the specification and the written description rejection of claims 32 and 41 is accordingly respectfully requested.

Rejection Under 35 USC §112, Second Paragraph

Claims 27, 28, 33, 34 and 36-41 have been rejected under 35 USC §112, second paragraph as being indefinite. This rejection is respectfully traversed.

The Office Action asserts that claims 27 and 36 each depend upon a canceled claim. However, these claims have been amended to not depend upon a canceled.

The Office Action also raises an issue regarding the extending the primer step c) in claim 33. It is noted that this step is a cyclical step requiring at least one repetition. The wording of this step has accordingly been amended so that this is more clear.

The claims are thus clear, definite and have full antecedent basis.

This rejection is believed to be overcome, and withdrawal thereof is respectfully requested.

Rejections Based on QUAKE et al.

Claims 19-25, 31, 33, 34, 38, 40 and 42 have been rejected under 35 USC §103(a) as being unpatentable over QUAKE et al. (U.S. Publication No. 2002/0025529 in view of URDEA et al. (U.S. Patent 4,910,300). Claims 27, 28, 36 and 37 have been rejected under 35 USC §103(a) as being unpatentable over QUAKE et al. in view of URDEA et al., and further in view of WELLS et al. (*J. Biol. Chem.*, vol. 261, pages 6564-6570 (1986)). Claims 30 and 39 have been rejected under 35 USC §103(a) as being unpatentable over QUAKE et al. in view of URDEA et al., and further in view of UEMORI et al. (WO 97/24444 - taken form U.S. Patent 6,395,526) as evidenced by ATKINS (*Physical Chemistry*, 3rd Ed., Freeman and Col., New York 1986, page 278). Claims 32 and

41 have been rejected under 35 USC §103(a) as being unpatentable over QUAKE et al. in view of URDEA et al. and further in view of HYMAN (U.S. Patent 5,516,664). Claim 42 has been rejected under 35 USC §103(a) as being unpatentable over QUAKE et al. in view of URDEA et al., and further in view of WELLS et al.

These rejections are respectfully traversed.

The present invention pertains to a method for determining the sequence of a nucleic acid molecule. After providing a single-stranded form of the nucleic acid molecule, the primer is hybridized to the single-stranded form to form a template/primer complex. Then the primer is iteratively extended by the addition of a polymerase and a mixture of at least one nucleotide and at least one labeled derivative of the at least one nucleotide.

The independent claims of the present invention set forth that the at least one nucleotide is within the range of 1-50 mole-%. The utilization of this range avoids quenching and intra-molecular thiol group formation, while still allowing for efficient detection.

The important aspects of the present invention include the labeled derivative being formed from fluorophore linked to the nucleotide via a cleavable link formed from a disulfide bond.

QUAKE et al. pertain to analyzing polynucleotide sequences. Paragraphs 0192 to 0194 of QUAKE et al. discuss the removal of blocking groups and labels via photocleavable or

enzymatically cleavable reagents which may or may not have a blocking function. Paragraph 0194 of QUAKE et al. teaches the use of 3'-5' exonuclease. However QUAKE et al. does not teach the type of nucleotides used in the present invention.

Paragraph 0179 of QUAKE et al. discusses the use of unlabeled nucleotides. However, QUAKE et al. (see, e.g., claim 1) restricts their use to a "microfabricated synthesis channel." Claims 34-40 of QUAKE et al. refer to cyclic incorporation of fluorescently-labeled nucleotides in a mixture where the label is removed by photobleaching.

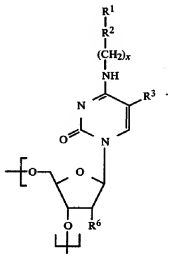
Now consider this restriction to a microfabricated synthesis channel in QUAKE et al. in light of the 1-50 mole-% labeled nucleotide of the present invention, which range avoids quenching and intra-molecular thiol group formation, while still allowing for efficient detection. The Official Action refers to paragraph 0179 of QUAKE et al., which should be considered in its entirety to understand its teachings:

> *[0179] In some applications of the present invention, a combination of labeled and non-labeled nucleotides are used in the analysis.* ***Because there are multiple copies of each template molecule immobilized on the surface of the synthesis channel, a small percentage of labeled nucleotides is sufficient for detection by a detection device*** *(see below). For example, for fluorescently labeled nucleotides, the percentage of labeled nucleotide can be less than 20%, less than 10%, less than 5%, less than 1%, less than 0.1%, less than 0.01%,*

or even less than 0.001% of the total labeled and unlabeled nucleotides for each type of the nucleotides. (Emphasis added).

That is, in QUAKE et al. the low amount of labeled nucleotides is used only because of the efficiency caused by the immobilization on the synthesis channel. There is no teaching or suggestion in QUAKE et al. of a percentage range of labeled nucleotide to address the problems of quenching and intra-molecular disulfide bond formation characteristic of a fluorophore-nucleotide with a disulphide linker.

Indeed, there is no teaching or inference at all of a fluorophore-nucleotide with a disulphide linker in QUAKE et al. To address this deficiency the Official Action refers to column 8 of URDEA et al., which discusses a polynucleotide probe of the Formula 13:



Formula 13

where R^1 is a reactive group derivatized with a detectable label and R^2 is an optional linking moiety that can be an amide, thioether of disulfide linkage.

However, URDEA et al. is directed to making nucleic acid probes (see, e.g., Title and Abstract). The disulfide bond in URDEA et al. is merely one out of a list of linkage possibilities (amide, thioether of disulfide linkage), and there is no recognition in URDEA et al. of the specific problems of quenching and intra-molecular thiol group formation that the present invention addresses.

That is, the independent claims of the present invention set forth the combination of a fluorophore-nucleotide with a disulphide linker. This can be in the presence of normal, non-labeled nucleotides (see claims 20 and 21). The present invention is not restricted to the use of a "microfabricated synthesis channel" which necessitates the use of a low percentage of labeled nucleotide. Rather, the present invention can use any amount up to 50 mole-% that accomplishes the elimination of quenching and intra-molecular thiol group formation.

The other applied art references fail to address the deficiencies of QUAKE et al. and URDEA et al. discussed above.

One of ordinary skill and creativity would fail to produce a claimed embodiment of the present invention from a knowledge of QUAKE et al. and URDEA et al., and a *prima facie* case of unpatentability has thus not been made.

Further (and as has been discussed in the previous response), the present invention displays unexpected results that would rebut any unpatentability that could be alleged. These unexpected results can be observed in the data shown in Figures 5-8 of the present invention, reproduced below.

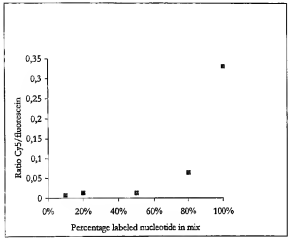


Figure 5.

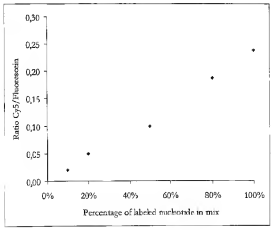


Figure 6.

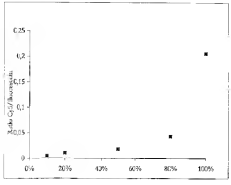


Figure 7.

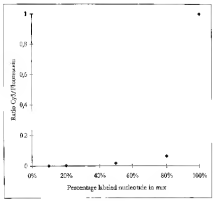


Figure 8

The results in Figures 5-8 show the selectivity of the polymerase for labeled against non-labeled nucleotides. There are clear differences in how the polymerase accepts the different Cy5-SS-nucleotides, in particular between U* and G*. The nodes at the claimed 50% limitation can be clearly observed.

That is, there is a stable regime up to 50%, which is a result of the elimination of quenching and intra-molecular thiol group formation. There is clearly no inference that this result can be achieved in the applied art references.

The advantages of the present invention are thus clear.

These rejections are believed to be overcome, and withdrawal thereof is respectfully requested.

Conclusion

It is believed that the rejections have been overcome, obviated or rendered moot, and no issues remain. The Examiner is accordingly respectfully requested to place the application in condition for allowance and to issue a Notice of Allowability.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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APPENDIX:

- typical specification sheets for these materials, which clearly set forth the chemical constituents of NP-40, Tween 20 and Triton X-100